

Figure 1. Nucleotide (SEQ ID No:1) and amino acid (SEQ ID No:2) sequences of the gene encoding the N. meningitidis strain 608B NspA protein.

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1      M K K A L A T L I A L A L P A A A L A E
1      ATGAAAAAAGCACTTGCCACACTGATTGCCCTCGCTCTCCCGGCCGCCGCACTGGCGGAA
21     G A S G F Y V Q A D A A H A K A S S S L
61     GGCGCATCCGGCTTTTACGTCCAAGCCGATGCCGCACACGCAAAGCCTCAAGCTCTTTA
41     G S A K G F S P R I S A G Y R I N D L R
121    GGTTCTGCCAAAGGCTTCAGCCCGCGCATCTCCGCAGGCTACCGCATCAACGACCTCCGC
61     F A V D Y T R Y K N Y K A P S T D F K L
181    TTCGCCGTCGATTACACGCGCTACAAAACTATAAAGCCCCATCCACCGATTTCAAACTT
81     Y S I G A S A I Y D F D T Q S P V K P Y
241    TACAGCATCGGCGCGTCCGCCATTTACGACTTCGACACCCAATCGCCCGTCAAACCGTAT
101    L G A R L S L N R A S V D L G G S D S F
301    CTCGGCGCGCGCTTGAGCCTCAACCGCGCCTCCGTCGACTTGGGCGGCAGCGACAGCTTC
121    S Q T S I G L G V L T G V S Y A V T P N
361    AGCCAAACCTCCATCGGCCTCGGCGTATTGACGGGCGTAAGCTATGCCGTTACCCCGAAT
141    V D L D A G Y R Y N Y I G K V N T V K N
421    GTCGATTTGGATGCCGGCTACCGCTACAAC TACATCGGCAAAGTCAACACTGTCAAAAAC
161    V R S G E L S V G V R V K F * (SEQ ID No:2)
481    GTCCGTTCCGGCGAACTGTCCGTCGGCGTGCGCGTCAAATTCTGA (SEQ ID No:1)

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Figure 2. 3-D model of the meningococcal NspA protein. This model was developed from the crystal structure of the refolded *E. coli* OmpA (PDB: 1QJP) [Pautsch, A. and GE Schulz, J. Mol. Biol., 298, p. 273 (2000)] using Swiss-Pdb Viewer [Guex, N. and MC Peitsch, Electrophoresis, 18, p. 2714 (1997)]. The eight transmembrane β -strands are connected with three tight turns (T) on the periplasmic side and four surface-exposed loops (L1, L2, L3, L4) on the outer surface of the bacteria. The amino acid residues, which interact with the membrane interphase are represented as balls and sticks. This figure was prepared using 3D-Mol Viewer from vector NTI suite 7.0 (InforMax, Inc.).

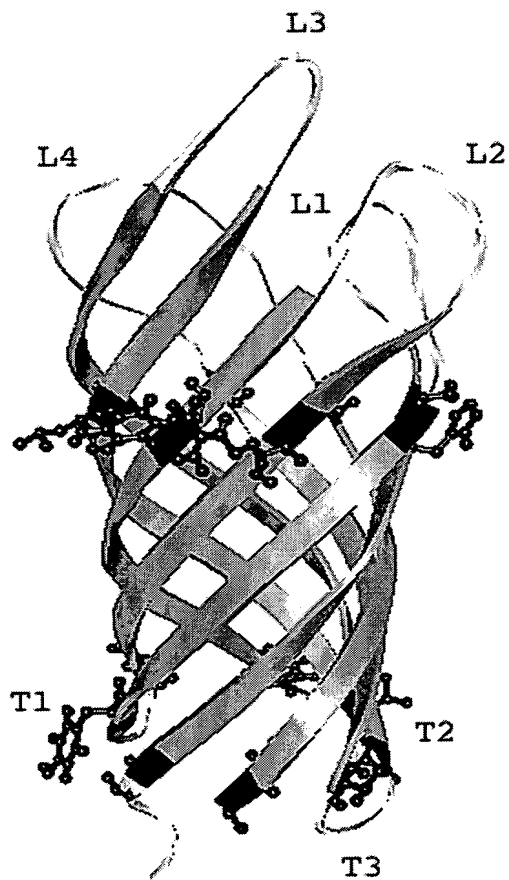


Figure 3. Evaluation by flow cytometry of the accessibility of NspA-specific MAbs at the surface of two serogroup B meningococcal strain 608B (B:2a:P1.2:L3), CU385 (B:4:P1.15:L3,7,9), one serogroup A strain F8238 (A:4,21) and one serogroup C strain C11 (NT:P1.1:L3,7,9). Exponentially growing meningococcal cells were sequentially incubated with NspA-specific or control MAbs, followed by FITC-conjugated anti-mouse immunoglobulin secondary antibody. The bactericidal activity of each MAb is presented as the concentration of antibody resulting in a 50% decrease of CFU per mL after 60 min of incubation compared to control CFU: ++, between 0.5-49 μ g of antibody/mL; +, between 50-99 μ g of antibody/mL; - no bactericidal activity at > 100 μ g of antibody/mL.

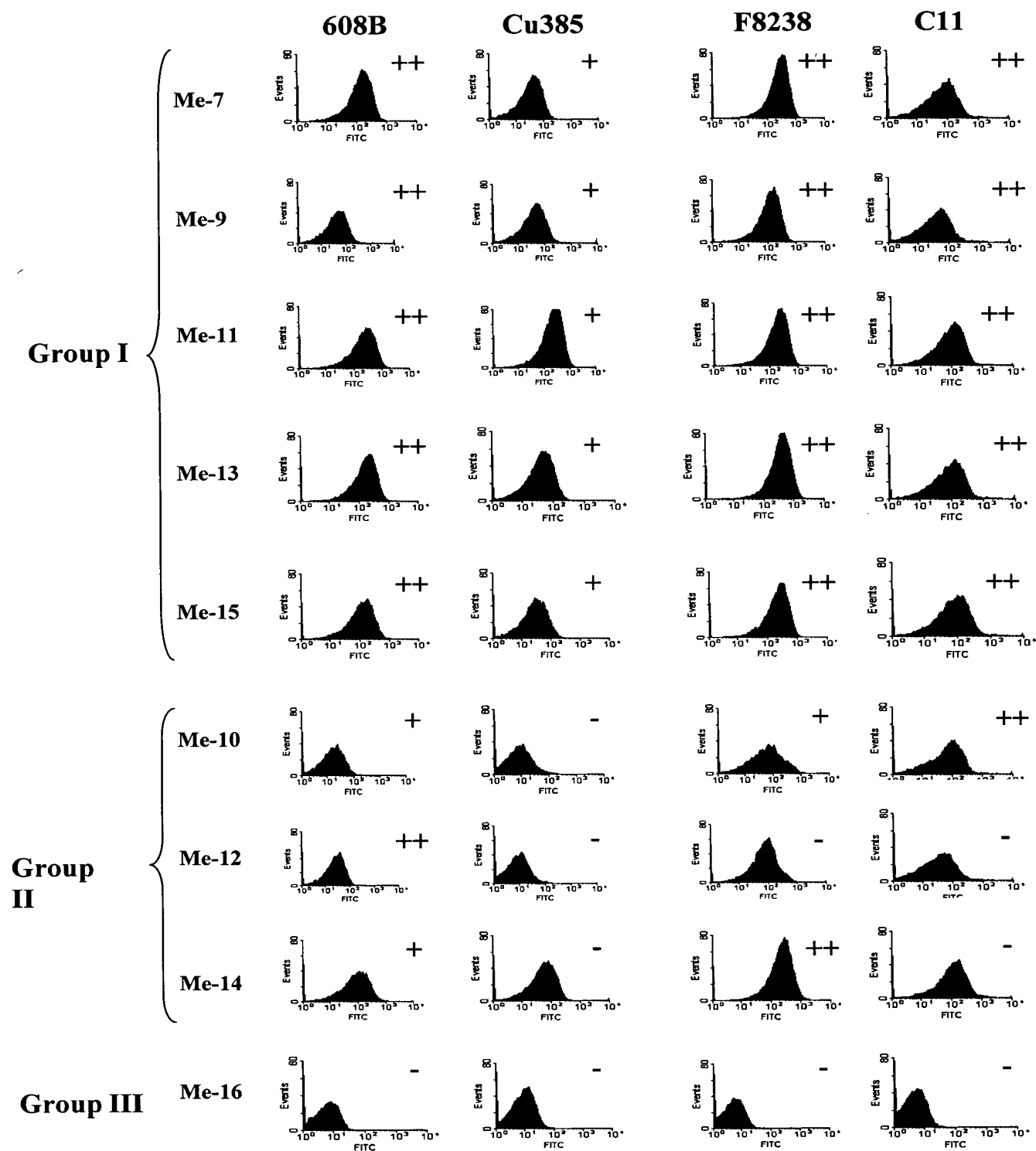


Figure 4. Evaluation of the binding of polyclonal anti-NspA rabbit antisera to *Neisseria meningitidis* strains 608B (B:2a:P1.2), BZ198 (B:NT:P-), S3446 (B:14:P1.23,14) and H355 (B:15:P1.15), as determined by indirect fluorescence flow cytometry. Rabbits were immunized with 100 µg of rNspA incorporated into different liposome formulations. Exponentially growing meningococcal cells were sequentially incubated with pre-bleed or hyperimmune sera, followed by fluorescein isothiocyanate (FITC)-conjugated anti-rabbit immunoglobulin secondary antibody. All sera were tested at a dilution of 1:20. In each graph, the left peak represents the binding of pre-bleed rabbit serum, while the right peak represents the binding of the corresponding hyperimmune serum against intact meningococcal cells.

